WORK PLAN AND METHODOLOGY

WORK PLAN

1. Review of literature.
2. Plant material collection and authentification.
3. Morpho-anatomical studies of leaves, stem and root of *Urena lobata* Linn.
4. Plant Extraction.
5. Qualitative phytochemical analysis.
6. Antioxidant activity.
7. Wound healing activity.
8. Anti cancer activity.

METHODOLOGY

Review of literature

Literature review is a continuous process from starting of the project to the final stage of the project. For literature review sources used are journals which includes national and international, patent, internet, books, standards like I.P, U.S.P, extra pharmacopeia WHO guidelines etc.

Plant material collection and authentification

The plant materials *Urena lobata* Linn collecting from the Herbal Garden Division of Kerala Ayurveda Ltd, Aluva, Kerala, India.

Morpho-anatomical studies of leaves stem and root of *Urena lobata* Linn

Preparation of specimen

The specimen for the plant will be prepared by standard procedure[40].

Sectioning

Sectioning is done by with help of Senior Precision Rotary microtome (MT-1090A, by WESWOX, India) dewaxing of the sections was done by normal procedure[41,42].

Photomicrographs

Microscopic photographs of tissues of different magnifications taken Nikon lab photo 2 microscopic units. Since these structures have birefringent property, under polarized light they appear bright against dark background[43, 44].

Plant Extraction

Methanolic Extraction and **Aqueous Extraction**

The coarse material is labeled and submit to Green Chem laboratory, Domlur, Bangalore, India for extraction [45] .

Qualitative phytochemical analysis

Qualitative tests for the identification of various phytoconstituents such as alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenol and flavonoids according to standard procedure[46] .

Antioxidant activity of the methanolic and aqueous extracts of *Urena lobata* Linn by DPPH method

- Preparation of the DPPH solution
- Preparation of the test solution

The radical scavenging activity (**percentage inhibition and IC_{50} value**) was calculated by the following formula[47].**Percentage Inhibition =** $\frac{A_C - A_S}{A_C} \times 100$

Wound healing activity of the methanolic extract of *Urena lobata* Linn.

- **Experimental Animals**

Albino wistar rats weighing 200-220 g and Albino mice 20-30 g was procured from Biogen, Bangalore, India.

- **Acute Toxicity Studies (LD_{50})**

Dissolve methanolic extract of *Urena lobata* Linn in saline/water. The doses selecting according to the OECD guidelines 423[48]

- **Drug Formulations**

For topical application, 10 and 20 g of extract taken with 90 and 80 g of 2% sodium alginate[49] and for oral administration the extract is dissolved in normal saline

- **Wound Healing Activity**

Wound healing activity was studied using four models [50, 51, 52,53].

- Excision,
- Incision,
- Burn wound
- Dead space wound model

- Hydroxyproline estimation
- Histopathological Study

Antitumor activity of methanolic and aqueous extracts of *Urena lobata* Linn

- Induction of Experimental Tumor
- Treatment Schedule
- **Evaluation of antitumor activity**

Antitumor activity was measured by the following [54,55]

- Mean Survival Time (MST)
- Percentage of Increase Life Span (% ILS)
- Tumour volume
- Viable and non viable cell count
- Tumor weight

Haematological Parameters

- White blood cell (WBC) count
- Red blood cell (RBC) count
- Haemoglobin (Hb)

Statistical Analysis

Data is analyzing by one way ANOVA followed by Dunnett's test using Graph pad prism software.